bjh review

Antiangiogenesis in haematological malignancies

William W. Li,[1] Michelle Hutnik[1] and Gerald Gehr[1,2,3]

[1]The Angiogenesis Foundation, Cambridge, MA, [2]Department of Medicine, Dartmouth Medical School, Lebanon, NH, and [3]Department of Hematology-Oncology, Department of Veterans Affairs Medical Center, Manchester, NH, USA

Summary

Angiogenesis, the growth of new capillary blood vessels, is a central regulator of cancer growth, and a validated target for cancer therapy. The antiangiogenic agents in clinical use target one or more cellular pathways involved in the cascade of vascular growth. In haematological malignancies, angiogenesis occurs within a bone marrow ecosystem comprised of closely apposed malignant cells, endothelial cells, pericytes, fibroblasts, endothelial progenitor cells, dendritic cells, and extracellular matrix. Inhibition of angiogenesis therefore blocks not only the delivery of oxygen and micronutrients to cancer cells, but also disrupts the interdependency of these cellular players and the paracrine effects they exert to maintain the malignant phenotype. Agents such as thalidomide, lenalidomide, bortezomib, and bevacizumab, have demonstrated clinical activity in myeloma, myelodysplastic syndrome, and leukaemias. In leukaemia, vascular endothelial growth factor (VEGF) is emerging as a compelling biological target for therapy, as well as a potential predictive marker for disease relapse. Initial clinical studies suggest that the anti-VEGF strategies may advance the primary, sequential or adjunctive treatment for leukaemia, and establish the basis for other potential anti-angiogenic strategies in haematological malignancies.

Keywords: angiogenesis, antiangiogenesis, haematogenous malignancies, vascular endothelial growth factor, leukaemia.

Angiogenesis, the growth of new capillary blood vessels, is a central regulator of cancer growth. The tumor vasculature delivers oxygen and micronutrients to proliferating cancer cells, and both tumor and endothelial cells release autocrine and paracrine signals that influence their microenvironment. The concept of 'antiangiogenesis' was pioneered in 1971 by Judah Folkman based on his observation that experimental solid tumors restricted from a blood supply were unable to expand (Folkman, 1971, 2007). Subsequently, the identification of specific angiogenic factors, such as vascular endothelial growth factor (VEGF), released by cancer cells enabled the development of targeted therapies to inhibit angiogenesis in cancer patients. In 2003, bevacizumab, an anti-VEGF monoclonal antibody, became the first antiangiogenic therapy to be validated as a cancer therapy, heralding the arrival of a fourth modality for cancer treatment, after surgery, radiation, and chemotherapy. A growing number of antiangiogenic agents, including tyrosine kinase inhibitors (sunitinib; sorafenib), anti-cytokine drugs (thalidomide; lenalidomide), and a proteosome inhibitor (bortezomib) (see Table I), have now entered clinical practice, and more than 100 agents are undergoing clinical or preclinical development.

The process of angiogenesis in haematological malignancies shares similarities with neovascularization of solid tumors. Endothelial cells within pre-existing venules are activated, proliferate, migrate, and form vascular tubes. While aberrant compared to normal physiological blood vessels, malignant angiogenesis in bone marrow recapitulates developmental vessel formation with arterial-venous differentiation, vascular maturation, and the recruitment of endothelial progenitor cells that reside within bone marrow vascular niches. Complex and as yet poorly characterized interactions occur between the vasculature and normal and malignant cells, the extracellular matrix, growth factors and cytokines, in the bone marrow. These interactions underlie the basis for antiangiogenic therapy in haematological disease.

This review will discuss antiangiogenic therapy in the context of haematological malignancies, with a focus on leukaemia. The activity of the three antiangiogenic agents (thalidomide, lenalidomide, and bortezomib) in their approved indications (multiple myeloma and myelodysplastic syndromes) will be presented as proof of principle of the efficacy of antiangiogenic therapies in haematological malignancies. Their activity supports the mounting body of evidence of the key role that VEGF plays in the pathology of bone marrow angiogenesis in leukaemia.

Targeting angiogenesis in bone marrow

Increased bone marrow vascularity reflects angiogenesis induced by multiple myeloma, myelodysplastic syndromes (MDS), leukaemia, and other haematological cancers (Vacca *et al*, 1994; Perez-Atayde *et al*, 1997; Pruneri *et al*, 1999; Padro *et al*, 2000; Sezer *et al*, 2000; Keith *et al*, 2007). A large body of

Correspondence: William W. Li, The Angiogenesis Foundation, 124 Mount Auburn Street, Suites 200N, Cambridge, MA 02138, USA.
E-mail: wli@angio.org

doi:10.1111/j.1365-2141.2008.07372.x

Table I. FDA-approved antiangiogenic therapies.

Generic name (brand name: company)	Indication	Date approved (country)	Dosing schedule
Cancer treatments			
Bevacizumab (Avastin: Genentech)	Metastatic colorectal cancer	2/2004 (USA)	5 mg/kg i.v. every 2 weeks + 5-FU and Leucovorin or FOLFOX or FOLFIRI
			7·5 mg/kg i.v. every 3 weeks + CapeOX
	Metastatic non-small cell lung cancer – first line	10/2006 (USA)	15 mg/kg i.v. every 3 weeks + paclitaxel and carboplatin
	Metastatic breast cancer	3/2007 (EU)	10 mg/kg i.v. every 2 weeks + paclitaxel
Bortezomib (Velcade: Millennium)	Multiple myeloma – second line	5/2003 (USA)	3- to 5-second bolus 1·3 mg/m^2 i.v. twice weekly for 2 weeks followed by a 10-d rest period (21 d cycle)
	Mantle cell lymphoma – second line	12/2006 (USA)	1·3 mg/m^2 i.v. 3- to 5-second bolus twice weekly for 2 weeks followed by a 10-d rest period (21 d cycle)
Cetuximab (Erbitux: ImClone/BMS)	Metastatic colorectal cancer – second line	2/2004 (USA)	400 mg/m^2 initial loading dose 250 mg/m^2 weekly
	Locally advanced or metastatic head and neck cancer	12/2005 (Switzerland)	400 mg/m^2 initial loading dose 250 mg/m^2 weekly with or without radiation
Endostar (rh-endostatin: Simcere Pharmaceuticals)	non-small cell lung cancer – first and second line	9/2005 (China)	7·5 mg/m^2 days 1 to 14 in 3 week cycle + vinorelbine + cisplatin
Erlotinib (Tarceva: Genentech/OSI/Roche)	Locally advanced or metastatic non-small cell lung cancer – second line	11/2004 (USA)	150 mg/d
	Pancreatic cancer – first line	11/2005 (USA)	100 mg/d + gemcitabine
Lenalidomide (Revlimid: Celgene)	Multiple myeloma – second line	6/2006 (USA)	25 mg/d capsule on Days 1 to 21 of repeated 28-d cycles + dexamethasone
	Deletion 5q myelodysplastic syndrome	12/2005 (USA)	10 mg/d capsule on Days 1 to 21 of repeated 28-d cycles
Sorafenib (Nexavar: Bayer/Onyx)	Advanced renal cell carcinoma – second line	12/2005 (USA)	400 mg twice per day
	Unresectable hepatocellular carcinoma	10/2007 (EU)	400 mg twice per day
Sunitinib Malate (Sutent: Pfizer)	Advanced renal cell carcinoma – first line	1/2006 (USA)	50 mg once daily for 4 weeks, followed by 2 weeks off treatment
	Gastrointestinal stromal tumor	1/2006 (USA)	50 mg once daily for 4 weeks, followed by 2 weeks off treatment
Thalidomide (Thalomid: Celgene)	Erythema nodosum leprosum	6/1998 (USA)	100–400 mg/d
	Multiple myeloma	12/2003 (Australia)	200 mg/d + dexamethasone

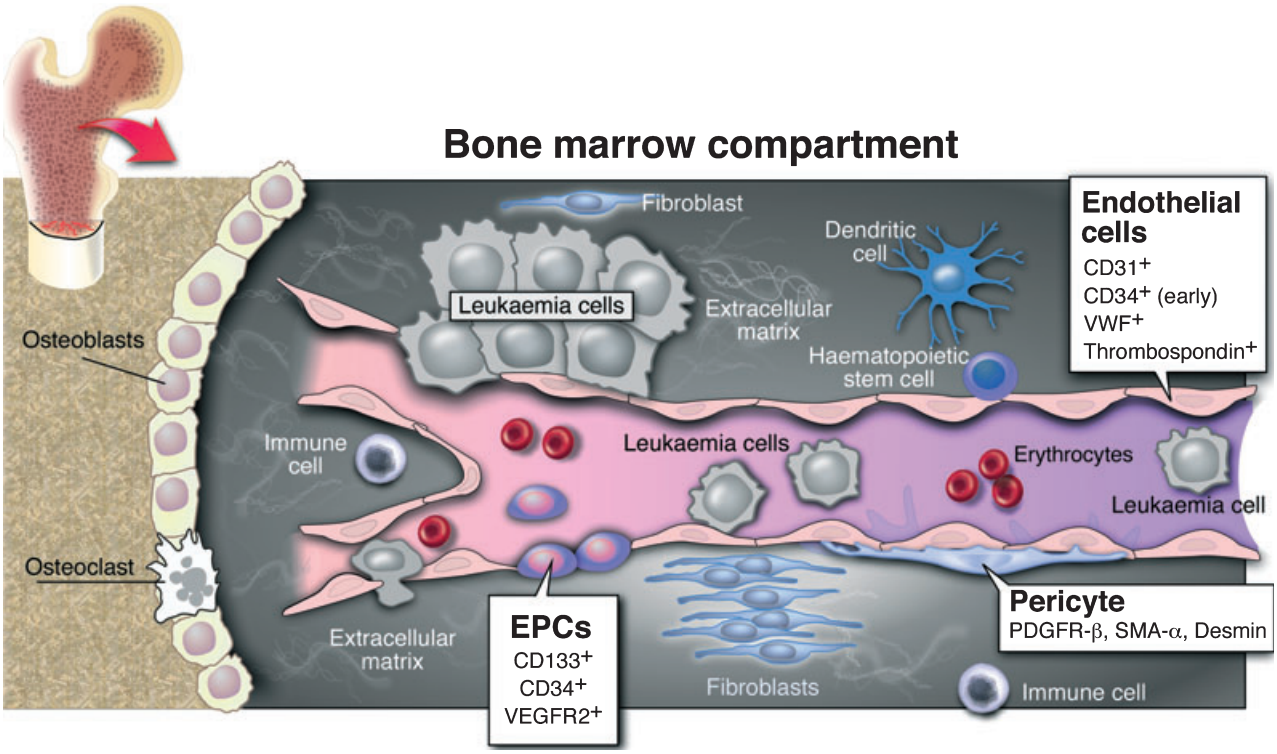

Fig 1. Antiangiogenic therapies in haematological cancers can target multiple cells and pathways in the bone marrow microenvironment, comprised of extracellular matrix (fibronectin, laminin, collagen) tightly interdispersed with vascular endothelial cells, pericytes, hematopoietic stem cells, endothelial progenitor cells (EPCs), immune cells (including Natural Killer cells, T lymphocytes, and monocytes), stromal cells (fibroblasts), dendritic cells, osteoblasts, and osteoclasts. Antiangiogenic agents interfering with one cell or pathway can disrupt other interdependent cells and pathways.

evidence links the extent of neovascularization observed in bone marrow biopsy specimens with disease burden, prognosis, or treatment outcome (Rajkumar *et al*, 2002).

Physiological angiogenesis in the bone marrow is induced and maintained by the balanced interplay of cells, cytokines and growth factors within a complex ecosystem in which endothelial cells and pericytes proliferate in concert with fibroblasts, dendritic cells, inflammatory cells, and haematopoietic stem cells. Malignant cells in the bone marrow upset this balance by producing and responding to the same factors, thereby stimulating angiogenesis, leading to increased vascularity. (see Fig 1). Several factors, including interleukin-6, granulocyte-macrophage colony-stimulating factor and VEGF, have autocrine and paracrine effects acting on multiple cell types. The proliferation of bone marrow capillaries contributes to the interdependency of these cell populations, and their interruption by antiangiogenic agents interferes with cell-cell, cell-matrix, and cell-cytokine/growth factor interactions in a fashion distinct from that observed in solid tumors.

A growing number of agents that inhibit angiogenesis are demonstrating clinical activity in haematological cancers. Thalidomide, an immunomodulatory antagonist of tumour necrosis factor-alpha, inhibits endothelial cell activation by VEGF, basic fibroblast growth factor (bFGF), and other pro-angiogenic factors (D'Amato *et al*, 1994). Lenalidomide is a potent thalidomide analog that, like thalidomide, has a complex mechanism of action that includes antiangiogenic activity.

Bortezomib, a proteosome inhibitor and antagonist of the antiapoptotic nuclear factor κ-B pathway, induces endothelial cell apoptosis and represses hypoxia-inducible factor 1 and VEGF production (Williams *et al*, 2003; Shin *et al*, 2008). Bevacizumab is a monoclonal antibody that neutralizes VEGF and is highly active in numerous cancers (Ferrara *et al*, 2004).

The clinical studies of haematological cancers in which these agents have demonstrated activity will be reviewed.

Multiple myeloma

Thalidomide was the first angiogenesis inhibitor to demonstrate clinical efficacy in multiple myeloma (D'Amato *et al*, 1994; Anargyrou *et al*, 2008). Specifically in myeloma, thalidomide down-regulates VEGF secretion from bone marrow endothelial cells obtained from patients with active disease. In a landmark Phase 2 clinical trial, 169 previously treated patients with refractory myeloma received thalidomide monotherapy (Singhal *et al*, 1999; Barlogie *et al*, 2001). Partial response, defined as 50% paraprotein reduction, was achieved in 30% of patients, and 14% achieved a complete or nearly complete remission. The survival rate at 2 years was 48%. These results led to many subsequent clinical studies of thalidomide in myeloma, leading ultimately to Federal Drug

Administration (FDA) approval of the drug in 2006, for the treatment of newly diagnosed multiple myeloma, in combination with dexamethasone. In the pivotal Phase 3 trial, the response rate in patients receiving thalidomide plus dexamethasone was 63% compared to 41% with dexamethasone alone ($P = 0{\cdot}0017$) (Rajkumar *et al*, 2006). Long-term outcome measures, including time-to-progression (TTP) and progression-free survival (PFS), were recently reported for a 470 patient randomized, placebo-controlled Phase 3 clinical trial of a similar protocol in newly diagnosed multiple myeloma, with comparable overall response rates (Rajkumar *et al*, 2008). Significant increases resulted in both median TTP (22·6 vs. 6·5 months; $P < 0{\cdot}001$) and median PFS (14·9 vs. 6·5 months; $P < 0{\cdot}001$) for the thalidomide plus dexamethasone group *versus* dexamethasone alone.

Lenalidomide, in combination with dexamethasone, is FDA approved for the treatment of multiple myeloma in patients who have received at least one prior therapy. This agent inhibits angiogenesis by antagonizing the production of VEGF in the bone marrow milieu (Richardson *et al*, 2002). A retrospective analysis of pooled data from two Phase 3 clinical trials (MM009 and MM010) enrolling a combined total of 692 patients with previously treated relapsed/refractory multiple myeloma demonstrated an improved response rate (59·2% vs. 22·5%; $P < 0{\cdot}001$) and increased median TTP (48·1 vs. 20·1 weeks; $P < 0{\cdot}001$) for patients treated with lenalidomide and dexamethasone, compared with dexamethasone alone (Wang *et al*, 2006; Dimopoulos *et al*, 2007; Weber *et al*, 2007). The toxicities of lenalidomide are less severe and less frequent than those of its analog, thalidomide.

Bortezomib is also FDA-approved for the treatment of myeloma that has relapsed after two prior treatments, or where resistance has developed following the last treatment. A first-in-class proteosome inhibitor, bortezomib inhibits VEGF secretion in endothelial cells derived from patients with myeloma (Roccaro *et al*, 2006). In a Phase 3 trial involving 669 myeloma patients treated with at least one prior therapy, bortezomib increased median TTP (6·2 months vs. 3·5 months; $P < 0{\cdot}0001$), improved overall survival, and increased response rate (38% vs. 18%; $P < 0{\cdot}0001$), compared with high-dose dexamethasone (Kane *et al*, 2006).

Myelodysplastic syndromes

In myelodysplastic syndromes, bone marrow dysfunction causes underproduction of erythrocytes, leucocytes, and platelets, leading to anemia, infection, or bleeding. The three-year survival rate of patients with severe MDS is as low as 35%, with mortality often caused by infection or hemorrhage (Ma *et al*, 2007). Transformation from MDS to acute myeloid leukaemia (AML) occurs in up to 40% of patients, and carries a poor prognosis. VEGF is overexpressed by immature myeloid cells in the bone marrow of patients with MDS, and this is associated with increased bone marrow vascularity and the presence of neoplastic cells (Bellamy *et al*, 2001).

An early clinical study of thalidomide in MDS resulted in a 56% hematological response, with some patients achieving transfusion independence (Strupp *et al*, 2002). Lenalidomide is FDA-approved for the treatment of transfusion-dependent anemia due to Low- or Intermediate-1-risk MDS associated with a deletion 5q cytogenetic abnormality (del 5q MDS) with or without additional cytogenetic abnormalities. In MDS, lenalidomide directly targets the malignant clone as well as the bone marrow microenvironment. Specifically for del 5q MDS, lenalidomide inhibits the growth of del 5q progenitor cells and upregulates SPARC (secreted protein acidic and rich in cysteine), which is an antiangiogenic, antiproliferative and anti-adhesive protein that regulates extracellular matrix interactions (Pellagatti *et al*, 2007). In a study of 43 MDS patients with transfusion-dependent or symptomatic anemia, 56% of the patients treated with lenalidomide exhibited a therapeutic response (List *et al*, 2005). For patients with del 5q MDS, there was an 83% response rate, with 75% of those achieving a complete cytogenetic remission. In a larger study of 148 patients with del 5q MDS, lenalidomide treatment resulted in a 76% response rate, with 45% of 85 evaluable patients achieving complete cytogenetic remission (List *et al*, 2006).

Leukaemia

The evidence that angiogenesis plays a pathophysiological role in leukemia has been well documented [for reviews, see (Bellamy *et al*, 1999; Fiedler *et al*, 1997; Folkman *et al*, 2001; Giles, 2002; Hussong *et al*, 2000; Kessler *et al*, 2007; List, 2001)] Angiogenesis in leukaemia is documented by increased bone marrow microvessel density (MVD), and increased expression of hypoxia-inducible factor 1α (HIF-1 α), multiple angiogenic factors (VEGF, bFGF, angiopoietin-2), soluble vascular endothelial growth factor receptors (Flt-1, KDR), and decreased expression of endogenous angiogenesis inhibitors, such as thrombospondin-1 (Dong *et al*, 2007; Frater *et al*, 2008). A clinicopathological study of 40 children with newly diagnosed, untreated acute lymphoblastic leukaemia (ALL) documented a sixfold increase in leukaemic bone marrow MVD compared to bone marrow from control children ($P \leq 0{\cdot}0001$) (Perez-Atayde *et al*, 1997). Human leukaemia cells (B-cell, T-cell ALL) injected into non-obese diabetic severe combined immunodeficiency (NOD/SCID) mice induced bone marrow neovascularization (Veiga *et al*, 2006). Likewise, bone marrow endothelial cells exposed to plasma derived from bone marrow of ALL patients underwent proliferation, migration, and tube formation, in contrast to lack of activity following exposure to bone marrow plasma from normal patients. ALL cells co-cultured with bone marrow endothelial cells significantly reduced leukaemia cell apoptosis, whereas in control conditions, cultured ALL cells underwent spontaneous cell death. Therefore, leukaemia cells induce the angiogenic phenotype, while endothelial cells promote leukaemia cell survival and expansion. Furthermore, leukaemic bone marrow vasculature exhibits abnormal features similar to those

characteristics of solid tumors. Myeloid leukaemia cells implanted into a cranial window of SCID mice induced neovascularization in which microvessels were tortuous and hyperpermeable, hallmarks of tumor angiogenesis (Schaefer *et al*, 2008).

The biological relationship between leukaemia and blood vessels is further evidenced by the homing of leukaemic cells to bone marrow vascular niches, where they take residence. In experimental mice injected with ALL cells, this homing is mediated by stromal-cell-derived factor 1 (SDF-1) and its receptor CXCR4 (Sipkins *et al*, 2005). CXCR4 is highly expressed on the surface of ALL cells, and on endothelial progenitor cells. The SDF-1 ligand is highly expressed by endothelial cells found in the bone marrow vascular ''hot spots'' or niches attracting ALL cells. *In vitro*, ALL cells also home towards bone marrow endothelial cells assembled in Matrigel, a classic assay for angiogenesis (Veiga *et al*, 2006).

VEGF as an angiogenesis target in leukaemia

As in other cancers, VEGF is a target for antiangiogenic agents in leukaemia. In addition to its well-defined effects on vascular endothelial cells, VEGF has direct and indirect effects on haematopoietic stem cells (HSC), endothelial progenitor cells, immune cells (including Natural Killer cells, T lymphocytes, and monocytes), dendritic cells, stromal cells, and in the case of neoplasia, malignant cells (Podar & Anderson, 2005). The VEGF pathway is involved in paracrine, autocrine, and juxtacrine signaling of multiple growth factors, and in regulating haematopoiesis by mediating HSC survival and differentiation (Gerber & Ferrara, 2003). VEGF inhibits dendritic cell maturation, modulates immune responses, influences bone homeostasis, and mediates progenitor cell recruitment and differentiation. VEGF is also an autocrine regulator of some cancer cells, (Dias *et al*, 2002; Vales *et al*, 2007) and is expressed in various leukaemia cell lines, including ALL, AML, acute T-cell leukaemia, chronic myeloid leukaemia (CML), B-cell chronic lymphocytic leukaemia (B-CLL), promyelocytic leukaemia, plasma cell leukaemia, as well as in leukemia blasts. Both internal and external autocrine VEGF loops have been found to mediate leukemia cell survival and proliferation (Santos & Dias, 2004). Therefore, VEGF directed therapy in leukaemia has multiple targets in the bone marrow milieu.

Circulating VEGF levels have been shown to have prognostic value in studies of pediatric and adult leukemia (Aguayo *et al*, 2002; Faderl *et al*, 2005; Avramis *et al*, 2006). Caution needs to be taken in viewing studies employing serum VEGF levels, as serum levels may overestimate true circulating VEGF levels because of the release of sequestered VEGF from platelets (Banks *et al*, 1998). However, comparison studies using serum levels involving control groups may still yield trends and valuable insights. In a study of newly diagnosed cases of pediatric ALL ($n = 31$), children with higher cellular VEGF levels at diagnosis had a poorer prognosis, with elevated VEGF levels correlating to a 10-fold decrease in the relapse-free interval (>10 years vs. 1·2 years) (Koomagi *et al*, 2001). Five of the study patients who relapsed had VEGF levels that were higher at relapse than upon initial diagnosis. In a clinical study (CCG-1962) of native *versus* pegylated-asparaginase therapy in standard-risk ALL pediatric patients ($n = 117$), high or increasing levels of VEGF serum during induction correlated with poorer survival and more frequent events (central nervous system or bone marrow relapse) (Avramis *et al*, 2006). By contrast, ALL children with low VEGF levels during induction experienced longer event-free survival. At the end of induction, high VEGF levels (>60 pg/l) correlated with worse survival compared to low VEGF levels (<60 pg/l) ($P < 0{\cdot}0001$). VEGF secreted by AML cells obtained at presentation was an independent prognostic factor for duration of relapse free survival in 47 children with newly diagnosed AML, following treatment using intensive chemotherapeutic protocols from the Dutch Childhood Leukaemia Study Group (de Bont *et al*, 2002). High VEGF secretion correlated with shorter relapse free survival, irrespective of cytogenetic abnormalities, white blood cell count, French-American-British (FAB) classification, risk assessment, and age at diagnosis.

Similar associations have been documented in adult leukaemia. In 58 adults with previously untreated AML, increased levels of plasma VEGF correlated with reduced survival ($P = 0{\cdot}02$) and lower complete remission rates ($P = 0{\cdot}004$) (Aguayo *et al*, 2002). Pretreatment cellular VEGF concentrations obtained from peripheral blood and bone marrow samples from newly diagnosed adult AML patients ($n = 99$) with high leucocyte and blast counts were prognostic in multivariant analysis (Aguayo *et al*, 1999). In another larger study of adult AML ($n = 133$), high plasma levels of soluble VEGF receptor-1 (sVEGFR1 or sFlt-1) was associated with more aggressive disease, while lower levels correlated with complete responses to treatment (Hu *et al*, 2004). Assessment of pretreatment bone marrow cellular levels of VEGF in chronic phase CML patients ($n = 148$) revealed an inverse relationship between VEGF levels and survival (Verstovsek *et al*, 2002). Taken together, these multiple lines of scientific, laboratory, and clinicopathological evidence support the rationale for VEGF-directed antiangiogenic therapy in leukaemia.

Clinical experience with antiangiogenic therapy in leukaemia

Several types of approved angiogenesis inhibitors have been tested in adult leukaemia patients: anti-cytokine agents, proteosome inhibitors, multikinase inhibitors, a monoclonal antibody, and mTOR (mammalian target of rapamycin) inhibitors. Each of these targets has been implicated in leukemia and are downstream (or upstream) in the VEGF signaling cascade.

Thalidomide has been clinically evaluated in advanced AML patients, both as monotherapy and combination with

chemotherapy (Steins *et al*, 2003; Barr *et al*, 2007). Although single-agent thalidomide has both antiangiogenic and anti-leukaemic activity in AML, its clinical efficacy is modest, with response rates of only between 6% and 24%. The limited responses coupled with toxicities associated with thalidomide, such as sedation, neuropathy, and constipation, have limited its development for leukaemia. The less toxic lenalidomide has activity as monotherapy in relapsed/refractory CLL patients. A small study ($n = 44$) showed an overall response rate of 32%, with 7% patients who achieved a complete response, 23% a partial response, and 2% a nodular partial remission (Ferrajoli *et al*, 2008). Bortezomib, administered to AML patients ($n = 31$) in combination with idarubicin and cytarabine led to complete responses in 61% of patients, with a good safety profile (Attar *et al*, 2008). Serious pulmonary and cardiac toxicities have been associated with bortezomib therapy, and significant precautions for its use in patients with pulmonary or pericardial disease are recommended.

Small molecule tyrosine kinase inhibitors are an important class of antiangiogenic agents. SU5416 inhibits the kinase signaling of VEGFR-1,-2, c-kit, and Flt3. This agent was studied in 43 patients with advanced AML demonstrating an overall response rate of 19%, with one complete morphological response lasting 2 months (Fiedler *et al*, 2003). Another multikinase angiogenesis inhibitor, PTK787, targets VEGFR-1,-2,-3, platelet-derived growth factor receptor (PDGFR), and c-kit signaling. PTK787 was studied in a Phase 1 clinical trial alone or in combination with cytosine arabinoside and daunorubicin (upfront or if monotherapy was ineffective) in patients with advanced MDS or AML. PTK787 monotherapy was administered (Arm 1; $n = 18$) in patients with primary refractory or relapsed AML. In a second arm of 45 patients with secondary AML, poor-prognosis de novo AML or advanced MDS, patients received PTK787 monotherapy (Arm 2M; $n = 35$), or combination therapy (Arm 2C; $n = 17$) upfront or following monotherapy. Although there were no responses in Arm 1, in Arm 2M there were two patients who achieved SD for 10–14 months. In Arm 2C, there were five complete remissions, two complete remissions (platelets not recovered), and one partial remission (Roboz *et al*, 2006).

Sunitinib is a multikinase inhibitor of VEGFR-1,-2, PDGFR, Flt3, and c-kit that is approved for renal cell carcinoma and gastrointestinal stromal tumors. It was evaluated in a Phase 1 clinical trial in refractory AML patients ($n = 15$) (Fiedler *et al*, 2005). In four patients with Flt3 mutations, all had morphologic or partial responses of short duration. Flt3 activation stimulates haematopoiesis, leukaemia cell proliferation, and endothelial cell proliferation (Drexler, 1996). Flt3 inhibition in leukaemia cell lines suppressed VEGF production in a dose-dependent fashion (Lopes de Menezes *et al*, 2005). Approximately one-third of AML patients have activating Flt3 mutations, which are associated with poor outcome. Some patients with non-mutated Flt3 also show evidence of Flt3 activation (Knapper, 2007). Several Flt3 inhibitors have been studied in AML clinical trials, exhibiting modest single agent activity. Combinatorial therapy trials, combining Flt3 inhibitors and cytotoxic agents, in AML are underway. Sorafenib, another multikinase agent, is in clinical trials for AML, ALL, CML, and MDS.

Bevacizumab, a partially-humanized IgG monoclonal antibody that neutralizes VEGF-A, is an approved antiangiogenic therapy for cancers of the colon, lung, and breast. Bevacizumab administered as monotherapy in patients with heavily-treated refractory AML ($n = 9$) resulted in a time-dependent reduction in VEGF expression observed by bone marrow immunohistochemistry, but without a clinical response (Zahiragic *et al*, 2007). This is consistent with the vast majority of clinical studies in refractory solid tumors in which single agent bevacizumab is inadequate to achieve improvement in clinical outcome (Quesada *et al*, 2007).

When bevacizumab was combined, however, into timed sequential therapy involving 1-β-D-arabinofuronosylcytosine (ara-C) and mitoxantrone, an improved overall response rate of 48% resulted, including complete responses (33%) and partial responses (14·5%) (Karp *et al*, 2004). In this Phase 2 study, ara-C was administered on day 1, followed by mitoxantrone (day 4), then bevacizumab (day 8). Serum VEGF levels decreased within 2 h following bevacizumab administration, and bone marrow MVD declined over the course of 7 d. Ara-C itself may be converted into an angiogenesis inhibitor by replacing the oxygen atom in the arabinose sugar ring with a sulphur atom to create the structural analog, T-ara-C (Roy *et al*, 2006).

Inhibitors of mTOR are another important class of anti-angiogenic agents. These include: deforolimus, everolimus, rapamycin (sirolimus), and temsirolimus (Giles & Albitar, 2005; Martelli *et al*, 2007). Rapamycin and related mTOR inhibitors inhibit endothelial cell VEGF expression, as well as VEGF-induced endothelial cell proliferation (Dormond *et al*, 2007). In a pilot study of six patients with imatinib-resistant CML, rapamycin induced major and minor leucocyte responses, with an observed decrease in *VEGFA* mRNA levels in circulating leukaemic cells (Sillaber *et al*, 2008). Another study of rapamycin in adult refractory/relapsed AML showed partial responses (>50% reduction in the absolute number of blood blasts or ≥0% reduction in the percentage of marrow blasts) and stable disease (Recher *et al*, 2005). Deforolimus has been studied in a Phase 2 trial in pretreated patients with various hematological malignancies, including ALL, AML, CLL, CML, MDS, agnogenic myeloid metaplasia, mantle cell lymphoma and T-cell leukaemia/lymphoma (Rizzieri *et al*, 2008). Overall, 40% of deforolimus-treated patients experienced haematological improvement or stable disease.

Other antiangiogenic agents under testing in adult leukaemias include AZD2171, enzastaurin, homoharringtonine, and UCN-01. Homoharringtonine, a myelosuppressive plant alkaloid isolated from the Plum yew tree *Cephalotaxus*, downregulates VEGF in leukaemic cells, and has demonstrated clinical activity, including induction of complete remissions, in

patients with relapsed CML (Quintas-Cardama *et al*, 2007). The activity of these agents, and their value in comparison to existing therapies, remains to be established in future well-designed clinical trials.

Future directions

Because endothelial cell targets exist across all cancer types, antiangiogenic agents that are validated as safe and effective in solid tumors are logical candidates for testing in haematological malignancies. In addition to bevacizumab, sunitinib, and sorafenib, these approved agents include cetuximab, everolimus, recombinant human endostatin, erlotinib, and temsirolimus. Clinicians treating haematological malignancies should give due consideration to the entire class of antiangiogenic agents as having potential utility in disease management.

Identifying new targets and understanding their mechanisms unique to pathological bone marrow angiogenesis will pave new approaches to antiangiogenic therapy (Nimer, 2008). Novel endothelial targets might include growth factors and their receptors, adhesion molecules, and endothelial-secreted cytokines. Targeted disruption of paracrine and autocrine loops of cytokine, and adhesion-mediated signaling pathways may further amplify the anti-cancer effect in leukaemia, myeloma, and MDS. Studies of the genetic and epigenetic makeup of patients who are best- or non-responders to specific antiangiogenic therapies will reveal additional clinical features important for optimizing this modality.

Although oncology agents are primarily aimed at adult cancers, paediatric haematological malignancies are particularly important opportunities for antiangiogenic therapy development. Anti-VEGF agents, in combination with antimetabolite or cytotoxic drugs, clearly have activity in paediatric leukemia. The overall safety profile of angiogenesis inhibitor drugs represents an important feature for application to children, especially if relapse or even secondary cancers can be prevented with long-term, adjuvant or maintenance treatment. Because bone marrow is a reservoir for haematopoietic stem and other progenitor cells, treatment specificity to disease pathways will be important for minimizing side effects, including normal growth and development. Ultimately, it should be possible to exploit the principles of antiangiogenic therapy to generate interventions that can be used to induce remissions and subsequently, to prevent relapse through long-term maintenance therapy. The successful development of this application of antiangiogenesis would validate Folkman's original hypothesis of maintaining cancer in a permanent state of dormancy, in the setting of haematological malignancies.

Acknowledgements

The authors would like to dedicate this review to the late Dr. Judah Folkman, who inspired the creation and mission of the Angiogenesis Foundation, and provided years of support and wisdom to our organization.

References

Aguayo, A., Estey, E., Kantarjian, H., Mansouri, T., Gidel, C., Keating, M., Giles, F., Estrov, Z., Barlogie, B. & Albitar, M. (1999) Cellular vascular endothelial growth factor is a predictor of outcome in patients with acute myeloid leukemia. *Blood*, **94,** 3717–3721.

Aguayo, A., Kantarjian, H.M., Estey, E.H., Giles, F.J., Verstovsek, S., Manshouri, T., Gidel, C., O'Brien, S., Keating, M.J. & Albitar, M. (2002) Plasma vascular endothelial growth factor levels have prognostic significance in patients with acute myeloid leukemia but not in patients with myelodysplastic syndromes. *Cancer*, **95,** 1923–1930.

Anargyrou, K., Dimopoulos, M.A., Sezer, O. & Terpos, E. (2008) Novel anti-myeloma agents and angiogenesis. *Leukaemia & Lymphoma*, **49,** 677–689.

Attar, E.C., De Angelo, D.J., Supko, J.G., D'Amato, F., Zahrieh, D., Sirulnik, A., Wadleigh, M., Ballen, K.K., McAfee, S., Miller, K.B., Levine, J., Galinsky, I., Trehu, E.G., Schenkein, D., Neuberg, D., Stone, R.M. & Amrein, P.C. (2008) Phase I and pharmacokinetic study of bortezomib in combination with idarubicin and cytarabine in patients with acute myelogenous leukemia. *Clinical Cancer Research*, **14,** 1446–1454.

Avramis, I.A., Panosyan, E.H., Dorey, F., Holcenberg, J.S. & Avramis, V.I. (2006) Correlation between high vascular endothelial growth factor-A serum levels and treatment outcome in patients with standard-risk acute lymphoblastic leukemia: a report from Children's Oncology Group Study CCG-1962. *Clinical Cancer Research*, **12,** 6978–6984.

Banks, R.E., Forbes, M.A., Kinsey, S.E., Stanley, A., Ingham, E., Walters, C. & Selby, P.J. (1998) Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *British Journal of Cancer*, **77,** 956–964.

Barlogie, B., Desikan, R., Eddlemon, P., Spencer, T., Zeldis, J., Munshi, N., Badros, A., Zangari, M., Anaissie, E., Epstein, J., Shaughnessy, J., Ayers, D., Spoon, D. & Tricot, G. (2001) Extended survival in advanced and refractory multiple myeloma after single-agent thalidomide: identification of prognostic factors in a phase 2 study of 169 patients. *Blood*, **98,** 492–494.

Barr, P., Fu, P., Lazarus, H., Kane, D., Meyerson, H., Hartman, P., Reyes, R., Creger, R., Stear, K., Laughlin, M., Tse, W. & Cooper, B. (2007) Antiangiogenic activity of thalidomide in combination with fludarabine, carboplatin, and topotecan for high-risk acute myelogenous leukemia. *Leukaemia & Lymphoma*, **48,** 1940–1949.

Bellamy, W.T., Richter, L., Frutiger, Y. & Grogan, T.M. (1999) Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. *Cancer Research*, **59,** 728–733.

Bellamy, W.T., Richter, L., Sirjani, D., Roxas, C., Glinsmann-Gibson, B., Frutiger, Y., Grogan, T.M. & List, A.F. (2001) Vascular endothelial cell growth factor is an autocrine promoter of abnormal localized immature myeloid precursors and leukemia progenitor formation in myelodysplastic syndromes. *Blood*, **97,** 1427–1434.

de Bont, E.S., Fidler, V., Meeuwsen, T., Scherpen, F., Hahlen, K. & Kamps, W.A. (2002) Vascular endothelial growth factor secretion is an independent prognostic factor for relapse-free survival in pediatric acute myeloid leukemia patients. *Clinical Cancer Research*, **8,** 2856–2861.

D'Amato, R.J., Loughnan, M.S., Flynn, E. & Folkman, J. (1994) Thalidomide is an inhibitor of angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, **91,** 4082–4085.

Dias, S., Shmelkov, S.V., Lam, G. & Rafii, S. (2002) VEGF(165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. *Blood*, **99,** 2532–2540.

Dimopoulos, M., Spencer, A., Attal, M., Prince, H.M., Harousseau, J.L., Dmoszynska, A., San Miguel, J., Hellmann, A., Facon, T., Foa, R., Corso, A., Masliak, Z., Olesnyckyj, M., Yu, Z., Patin, J., Zeldis, J.B. & Knight, R.D. (2007) Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. *New England Journal of Medicine*, **357,** 2123–2132.

Dong, X., Han, Z.C. & Yang, R. (2007) Angiogenesis and antiangiogenic therapy in hematologic malignancies. *Critical Reviews in Oncology/hematology*, **62,** 105–118.

Dormond, O., Madsen, J.C. & Briscoe, D.M. (2007) The effects of mTOR-Akt interactions on anti-apoptotic signaling in vascular endothelial cells. *Journal of Biological Chemistry*, **282,** 23679–23686.

Drexler, H.G. (1996) Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia*, **10,** 588–599.

Faderl, S., Do, K.A., Johnson, M.M., Keating, M., O'Brien, S., Jilani, I., Ferrajoli, A., Ravandi-Kashani, F., Aguilar, C., Dey, A., Thomas, D.A., Giles, F.J., Kantarjian, H.M. & Albitar, M. (2005) Angiogenic factors may have a different prognostic role in adult acute lymphoblastic leukemia. *Blood*, **106,** 4303–4307.

Ferrajoli, A., Lee, B.N., Schlette, E.J., O'Brien, S.M., Gao, H., Wen, S., Wierda, W.G., Estrov, Z., Faderl, S.H., Cohen, E.N., Li, C., Reuben, J.M. & Keating, M.J. (2008) Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood*, **111,** 5291–5297.

Ferrara, N., Hillan, K.J., Gerber, H.P. & Novotny, W. (2004) Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nature Reviews. Drug Discovery*, **3,** 391–400.

Fiedler, W., Graeven, U., Ergun, S., Verago, S., Kilic, N., Stockschlader, M. & Hossfeld, D.K. (1997) Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. *Blood*, **89,** 1870–1875.

Fiedler, W., Mesters, R., Tinnefeld, H., Loges, S., Staib, P., Duhrsen, U., Flasshove, M., Ottmann, O.G., Jung, W., Cavalli, F., Kuse, R., Thomalla, J., Serve, H., O'Farrell, A.M., Jacobs, M., Brega, N.M., Scigalla, P., Hossfeld, D.K. & Berdel, W.E. (2003) A phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia. *Blood*, **102,** 2763–2767.

Fiedler, W., Serve, H., Dohner, H., Schwittay, M., Ottmann, O.G., O'Farrell, A.M., Bello, C.L., Allred, R., Manning, W.C., Cherrington, J.M., Louie, S.G., Hong, W., Brega, N.M., Massimini, G., Scigalla, P., Berdel, W.E. & Hossfeld, D.K. (2005) A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. *Blood*, **105,** 986–993.

Folkman, J. (1971) Tumor angiogenesis: therapeutic implications. *New England Journal of Medicine*, **285,** 1182–1186.

Folkman, J. (2007) Is angiogenesis an organizing principle in biology and medicine? *Journal of Pediatric Surgery*, **42,** 1–11.

Folkman, J., Browder, T. & Palmblad, J. (2001) Angiogenesis research: guidelines for translation to clinical application. *Thrombosis and Haemostasis*, **86,** 23–33.

Frater, J.L., Kay, N.E., Goolsby, C.L., Crawford, S.E., Dewald, G.W. & Peterson, L.C. (2008) Dysregulated angiogenesis in B-chronic lymphocytic leukemia: morphologic, immunohistochemical, and flow cytometric evidence. *Diagnostic Pathology*, **3,** 16.

Gerber, H.P. & Ferrara, N. (2003) The role of VEGF in normal and neoplastic hematopoiesis. *Journal of Molecular Medicine*, **81,** 20–31.

Giles, F.J. (2002) The emerging role of angiogenesis inhibitors in hematologic malignancies. *Oncology (Williston Park)*, **16,** 23–29.

Giles, F.J. & Albitar, M. (2005) Mammalian target of rapamycin as a therapeutic target in leukemia. *Current Molecular Medicine*, **5,** 653–661.

Hu, Q., Dey, A.L., Yang, Y., Shen, Y., Jilani, I.B., Estey, E.H., Kantarjian, H.M., Giles, F.J. & Albitar, M. (2004) Soluble vascular endothelial growth factor receptor 1, and not receptor 2, is an independent prognostic factor in acute myeloid leukemia and myelodysplastic syndromes. *Cancer*, **100,** 1884–1891.

Hussong, J.W., Rodgers, G.M. & Shami, P.J. (2000) Evidence of increased angiogenesis in patients with acute myeloid leukemia. *Blood*, **95,** 309–313.

Kane, R.C., Farrell, A.T., Sridhara, R. & Pazdur, R. (2006) United States Food and Drug Administration approval summary: bortezomib for the treatment of progressive multiple myeloma after one prior therapy. *Clinical Cancer Research*, **12,** 2955–2960.

Karp, J.E., Gojo, I., Pili, R., Gocke, C.D., Greer, J., Guo, C., Qian, D., Morris, L., Tidwell, M., Chen, H. & Zwiebel, J. (2004) Targeting vascular endothelial growth factor for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential 1-beta-d-arabinofuranosylcytosine, mitoxantrone, and bevacizumab. *Clinical Cancer Research*, **10,** 3577–3585.

Keith, T., Araki, Y., Ohyagi, M., Hasegawa, M., Yamamoto, K., Kurata, M., Nakagawa, Y., Suzuki, K. & Kitagawa, M. (2007) Regulation of angiogenesis in the bone marrow of myelodysplastic syndromes transforming to overt leukaemia. *British Journal Haematology*, **137,** 206–215.

Kessler, T., Fehrmann, F., Bieker, R., Berdel, W.E. & Mesters, R.M. (2007) Vascular endothelial growth factor and its receptor as drug targets in hematological malignancies. *Current Drug Targets*, **8,** 257–268.

Knapper, S. (2007) FLT3 inhibition in acute myeloid leukaemia. *British Journal Haematology*, **138,** 687–699.

Koomagi, R., Zintl, F., Sauerbrey, A. & Volm, M. (2001) Vascular endothelial growth factor in newly diagnosed and recurrent childhood acute lymphoblastic leukemia as measured by real-time quantitative polymerase chain reaction. *Clinical Cancer Research*, **7,** 3381–3384.

List, A.F. (2001) Vascular endothelial growth factor signaling pathway as an emerging target in hematologic malignancies. *Oncologist*, **6**(Suppl. 5)**,** 24–31.

List, A., Kurtin, S., Roe, D.J., Buresh, A., Mahadevan, D., Fuchs, D., Rimsza, L., Heaton, R., Knight, R. & Zeldis, J.B. (2005) Efficacy of lenalidomide in myelodysplastic syndromes. *New England Journal of Medicine*, **352,** 549–557.

List, A., Dewald, G., Bennett, J., Giagounidis, A., Raza, A., Feldman, E., Powell, B., Greenberg, P., Thomas, D., Stone, R., Reeder, C., Wride, K., Patin, J., Schmidt, M., Zeldis, J. & Knight, R. (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. *New England Journal of Medicine*, **355,** 1456–1465.

Lopes de Menezes, D.E., Peng, J., Garrett, E.N., Louie, S.G., Lee, S.H., Wiesmann, M., Tang, Y., Shephard, L., Goldbeck, C., Oei, Y., Ye, H., Aukerman, S.L. & Heise, C. (2005) CHIR-258: a potent inhibitor of FLT3 kinase in experimental tumor xenograft models of human acute myelogenous leukemia. *Clinical Cancer Research*, **11,** 5281–5291.

Ma, X., Does, M., Raza, A. & Mayne, S.T. (2007) Myelodysplastic syndromes: incidence and survival in the United States. *Cancer*, **109,** 1536–1542.

Martelli, A.M., Tazzari, P.L., Evangelisti, C., Chiarini, F., Blalock, W.L., Billi, A.M., Manzoli, L., McCubrey, J.A. & Cocco, L. (2007) Targeting the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin module for acute myelogenous leukemia therapy: from bench to bedside. *Current Medicinal Chemistry*, **14,** 2009–2023.

Nimer, S.D. (2008) Myelodysplastic syndromes. *Blood*, **111,** 4841–4851.

Padro, T., Ruiz, S., Bieker, R., Burger, H., Steins, M., Kienast, J., Buchner, T., Berdel, W.E. & Mesters, R.M. (2000) Increased angiogenesis in the bone marrow of patients with acute myeloid leukemia. *Blood*, **95,** 2637–2644.

Pellagatti, A., Jadersten, M., Forsblom, A.M., Cattan, H., Christensson, B., Emanuelsson, E.K., Merup, M., Nilsson, L., Samuelsson, J., Sander, B., Wainscoat, J.S., Boultwood, J. & Hellstrom-Lindberg, E. (2007) Lenalidomide inhibits the malignant clone and up-regulates the SPARC gene mapping to the commonly deleted region in 5q- syndrome patients. *Proceedings of the National Academy of Sciences of the United States of America*, **104,** 11406–11411.

Perez-Atayde, A.R., Sallan, S.E., Tedrow, U., Connors, S., Allred, E. & Folkman, J. (1997) Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. *American Journal of Pathology*, **150,** 815–821.

Podar, K. & Anderson, K.C. (2005) The pathophysiologic role of VEGF in hematologic malignancies: therapeutic implications. *Blood*, **105,** 1383–1395.

Pruneri, G., Bertolini, F., Soligo, D., Carboni, N., Cortelezzi, A., Ferrucci, P.F., Buffa, R., Lambertenghi-Deliliers, G. & Pezzella, F. (1999) Angiogenesis in myelodysplastic syndromes. *British Journal of Cancer*, **81,** 1398–1401.

Quesada, A.R., Medina, M.A. & Alba, E. (2007) Playing only one instrument may be not enough: limitations and future of the anti-angiogenic treatment of cancer. *Bioessays*, **29,** 1159–1168.

Quintas-Cardama, A., Kantarjian, H., Garcia-Manero, G., O'Brien, S., Faderl, S., Estrov, Z., Giles, F., Murgo, A., Ladie, N., Verstovsek, S. & Cortes, J. (2007) Phase I/II study of subcutaneous homoharringtonine in patients with chronic myeloid leukemia who have failed prior therapy. *Cancer*, **109,** 248–255.

Rajkumar, S.V., Mesa, R.A. & Tefferi, A. (2002) A review of angiogenesis and anti-angiogenic therapy in hematologic malignancies. *Journal of Hematotherapy & Stem Cell Research*, **11,** 33–47.

Rajkumar, S.V., Blood, E., Vesole, D., Fonseca, R. & Greipp, P.R. (2006) Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. *Journal of Clinical Oncology*, **24,** 431–436.

Rajkumar, S.V., Rosinol, L., Hussein, M., Catalano, J., Jedrzejczak, W., Lucy, L., Olesnyckyj, M., Yu, Z., Knight, R., Zeldis, J.B. & Blade, J. (2008) Multicenter, randomized, double-blind, placebo-controlled study of thalidomide plus dexamethasone compared with dexamethasone as initial therapy for newly diagnosed multiple myeloma. *Journal of Clinical Oncology*, **26,** 2171–2177.

Recher, C., Beyne-Rauzy, O., Demur, C., Chicanne, G., Dos Santos, C., Mas, V.M., Benzaquen, D., Laurent, G., Huguet, F. & Payrastre, B. (2005) Antileukemic activity of rapamycin in acute myeloid leukemia. *Blood*, **105,** 2527–2534.

Richardson, P.G., Schlossman, R.L., Weller, E., Hideshima, T., Mitsiades, C., Davies, F., LeBlanc, R., Catley, L.P., Doss, D., Kelly, K., McKenney, M., Mechlowicz, J., Freeman, A., Deocampo, R., Rich, R., Ryoo, J.J., Chauhan, D., Balinski, K., Zeldis, J. & Anderson, K.C. (2002) Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. *Blood*, **100,** 3063–3067.

Rizzieri, D.A., Feldman, E., Dipersio, J.F., Gabrail, N., Stock, W., Strair, R., Rivera, V.M., Albitar, M., Bedrosian, C.L. & Giles, F.J. (2008) A phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. *Clinical Cancer Research*, **14,** 2756–2762.

Roboz, G.J., Giles, F.J., List, A.F., Cortes, J.E., Carlin, R., Kowalski, M., Bilic, S., Masson, E., Rosamilia, M., Schuster, M.W., Laurent, D. & Feldman, E.J. (2006) Phase 1 study of PTK787/ZK 222584, a small molecule tyrosine kinase receptor inhibitor, for the treatment of acute myeloid leukemia and myelodysplastic syndrome. *Leukemia*, **20,** 952–957.

Roccaro, A.M., Hideshima, T., Raje, N., Kumar, S., Ishitsuka, K., Yasui, H., Shiraishi, N., Ribatti, D., Nico, B., Vacca, A., Dammacco, F., Richardson, P.G. & Anderson, K.C. (2006) Bortezomib mediates antiangiogenesis in multiple myeloma via direct and indirect effects on endothelial cells. *Cancer Research*, **66,** 184–191.

Roy, A.M., Tiwari, K.N., Parker, W.B., Secrist, III, J.A., Li, R. & Qu, Z. (2006) Antiangiogenic activity of 4′-thio-beta-D-arabinofuranosylcytosine. *Molecular Cancer Therapeutics*, **5,** 2218–2224.

Santos, S.C. & Dias, S. (2004) Internal and external autocrine VEGF/KDR loops regulate survival of subsets of acute leukemia through distinct signaling pathways. *Blood*, **103,** 3883–3889.

Schaefer, C., Krause, M., Fuhrhop, I., Schroeder, M., Algenstaedt, P., Fiedler, W., Ruther, W. & Hansen-Algenstaedt, N. (2008) Time-course-dependent microvascular alterations in a model of myeloid leukemia in vivo. *Leukemia*, **22,** 59–65.

Sezer, O., Niemoller, K., Eucker, J., Jakob, C., Kaufmann, O., Zavrski, I., Dietel, M. & Possinger, K. (2000) Bone marrow microvessel density is a prognostic factor for survival in patients with multiple myeloma. *Annals of Hematology*, **79,** 574–577.

Shin, D.H., Chun, Y.S., Lee, D.S., Huang, L.E. & Park, J.W. (2008) Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FIH-mediated repression of hypoxia-inducible factor-1. *Blood*, **111,** 3131–3136.

Sillaber, C., Mayerhofer, M., Bohm, A., Vales, A., Gruze, A., Aichberger, K.J., Esterbauer, H., Pfeilstocker, M., Sperr, W.R., Pickl, W.F., Haas, O.A. & Valent, P. (2008) Evaluation of antileukaemic effects of rapamycin in patients with imatinib-resistant chronic myeloid leukaemia. *European Journal of Clinical Investigation*, **38,** 43–52.

Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeddis, J. & Barlogie, B. (1999) Antitumor activity of thalidomide in refractory multiple myeloma. *New England Journal of Medicine*, **341,** 1565–1571.

Sipkins, D.A., Wei, X., Wu, J.W., Runnels, J.M., Cote, D., Means, T.K., Luster, A.D., Scadden, D.T. & Lin, C.P. (2005) In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature*, **435,** 969–973.

Steins, M.B., Bieker, R., Padro, T., Kessler, T., Kienast, J., Berdel, W.E. & Mesters, R.M. (2003) Thalidomide for the treatment of acute myeloid leukemia. *Leukaemia & Lymphoma*, **44,** 1489–1493.

Strupp, C., Germing, U., Aivado, M., Misgeld, E., Haas, R. & Gattermann, N. (2002) Thalidomide for the treatment of patients with myelodysplastic syndromes. *Leukemia*, **16,** 1–6.

Vacca, A., Ribatti, D., Roncali, L., Ranieri, G., Serio, G., Silvestris, F. & Dammacco, F. (1994) Bone marrow angiogenesis and progression in multiple myeloma. *British Journal Haematology*, **87,** 503–508.

Vales, A., Kondo, R., Aichberger, K.J., Mayerhofer, M., Kainz, B., Sperr, W.R., Sillaber, C., Jager, U. & Valent, P. (2007) Myeloid leukemias express a broad spectrum of VEGF receptors including neuropilin-1 (NRP-1) and NRP-2. *Leukaemia & Lymphoma*, **48,** 1997–2007.

Veiga, J.P., Costa, L.F., Sallan, S.E., Nadler, L.M. & Cardoso, A.A. (2006) Leukemia-stimulated bone marrow endothelium promotes leukemia cell survival. *Experimental Hematology*, **34,** 610–621.

Verstovsek, S., Kantarjian, H., Manshouri, T., Cortes, J., Giles, F.J., Rogers, A. & Albitar, M. (2002) Prognostic significance of cellular vascular endothelial growth factor expression in chronic phase chronic myeloid leukemia. *Blood*, **99,** 2265–2267.

Wang, M., Knight, R., Dimopoulos, M., Siegel, D., Rajkumar, S.V., Facon, T., Yu, Z., Zeldis, J., Olesnyckyj, M. & Weber, D.M. (2006) Comparison of lenalidomide in combination with dexamethasone to dexamethasone alone in patients who have received prior thalidomide in relapsed or refractory multiple myeloma. *Journal of Clinical Oncology (Meeting Abstracts)*, **24,** 7522.

Weber, D.M., Chen, C., Niesvizky, R., Wang, M., Belch, A., Stadtmauer, E.A., Siegel, D., Borrello, I., Rajkumar, S.V., Chanan-Khan, A.A., Lonial, S., Yu, Z., Patin, J., Olesnyckyj, M., Zeldis, J.B. & Knight, R.D. (2007) Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. *New England Journal of Medicine*, **357,** 2133–2142.

Williams, S., Pettaway, C., Song, R., Papandreou, C., Logothetis, C. & McConkey, D.J. (2003) Differential effects of the proteasome inhibitor bortezomib on apoptosis and angiogenesis in human prostate tumor xenografts. *Molecular Cancer Therapeutics*, **2,** 835–843.

Zahiragic, L., Schliemann, C., Bieker, R., Thoennissen, N.H., Burow, K., Kramer, C., Zuhlsdorf, M., Berdel, W.E. & Mesters, R.M. (2007) Bevacizumab reduces VEGF expression in patients with relapsed and refractory acute myeloid leukemia without clinical antileukemic activity. *Leukemia*, **21,** 1310–1312.